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Overview of Herbicide Mechanisms of Action

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Commercial herbicides exhibit many different mechanisms of action. Several enzymes involved in biosynthesis of amino acids are sites of action for herbicides. A large number of different herbicide classes inhibit photosynthesis by binding to the quinone-binding protein, D-1, to prevent photosynthetic electron transfer. Several different types of herbicides apparently cause accumulation of photodynamic porphyrins by inhibiting protoporphyrinogen oxidase. Bipyridyliums and heteropentalenes cause the production of superoxide radicals by energy diversion from photosystem I of photosynthesis. Lipid synthesis is the site of action of a broad array of herbicides used in controlling monocot weeds. Herbicides of several classes apparently act by inhibiting mitosis through direct interaction with tubulin. Several other molecular sites of herbicide action are known. Despite a growing body of knowledge, the exact molecular sites of action of many herbicides are unknown. Some herbicides are known to have more than one site of action. Virtually all knowledge of herbicide structure-activity relationships is semiempirical. In addition to site of action structure-activity relationships, herbicide structure and chemical properties also strongly influence absorption, translocation, bioactivation, and environmental stability. Considering how little is known about all the potential sites of herbicide action, it is unlikely that during the next decade more than a relatively small number of site-specific herbicide structure-activity relationships will be developed.

Introduction

Herbicides represent an extremely broad array of chemical classes and, in turn, act at a large number of sites of metabolic function and energy transfer in plant cells. The diversity of sites of action is greater than that for insecticides, perhaps because of the relatively long time frame used in traditional screens for herbicides versus those for insecticides. To date, few, if any, herbicides have been produced by a completely nonempirical, structure-activity design process that is based on knowledge of the molecular site of action. In fact, the exact molecular site of only a few herbicide classes is known. Only those herbicides for which the molecular site is firmly established or for which a substantial amount of indirect data points to one site of action will be discussed in this review.

This summary is meant to impress the reader with the difficulty in predicting a molecular mechanism of action by analysis of the chemical structure of the herbicide molecule. At present, this can probably only be done by semiempirical knowledge of the quantitative structure-activity relationships for classes of herbicides having a known mode of action. However, determination of the mechanisms of action of new herbicide classes is still done by physiological and biochemical testing by even the most advanced herbicide discovery teams. The molecular site of action of several important herbicide classes is still unknown, despite considerable research. The capability of accurately predicting the herbicidal activity and site of action of a new chemical class without extensive laboratory studies would be worth tens of millions of dollars to the herbicide industry.

The mechanism of action of herbicides is summarized in more detail in several recent books (*1–3*). However, this field of research is advancing so rapidly that no recent book adequately covers the current status of this area. Due to its brevity, this review will provide only a limited description of the more completely understood herbicide mechanisms of action and an even more limited discussion of the generally poorly understood structure-activity relationships for herbicides active at these sites.

Modes of Action

Amino Acid Biosynthesis

Three enzymes of amino acid biosynthesis constitute important sites of herbicide action: 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSP synthase), acetolactate synthase (ALS, also known as acetohydroxyacid synthase), and glutamine synthetase (GS). These sites of action are firmly established as the primary sites of action of several important herbicide classes. Many other potential herbicide sites of action exist among the enzymes of amino acid biosynthesis, and there are several herbicides and potential herbicides that may directly affect these enzymes.

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EPSP synthase is an enzyme in the shikimate pathway (Fig. 1), the metabolic pathway that produces aromatic amino acids (i.e., phenylalanine, tyrosine, and tryptophan). It converts phosphoenolpyruvate and shikimate-3-phosphate to EPSP. The enzyme is nuclear-coded, but apparently functions primarily in the plastid, despite being fully active in the cytoplasm. Glyphosate [*N*-(phosphonomethyl)glycine] (Fig. 2) competitively inhibits the enzyme with respect to phosphoenolpyruvate with a K_i of 1 μM (*4*). Blockage of this enzyme results in massive accumulation of shikimate in affected plant tissue. This effect is exacerbated by deregulation of the shikimate pathway. The *in vivo* activity of 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase, an earlier enzyme in the shikimate pathway, is increased as a result of glyphosate inhibition of EPSP synthase (*5*). Glyphosate is the only compound known to inhibit EPSP synthase sufficiently to be a viable herbicide.

ALS is a key enzyme in the branched chain amino acid pathway (Fig. 3) that produces leucine, isoleucine, and valine. The enzyme catalyzes two reactions: condensation of two pyruvate molecules to produce CO_2 and α-acetolactate, a precursor of valine and leucine; and condensation of pyruvate and α-ketobutyrate to form CO_2 and 2-acetohydroxybutyrate, a precursor of isoleucine. Three different herbicide groups inhibit this enzyme; the sulfonylureas (*6*) (Fig. 2), the imidazolinones (*7*) (Fig. 2), and the 1,2,4-triazol [1,5*A*] pyrimidines (*7*). The first two of these groups have been commercialized, and the last group is being considered for commercialization.

Several sulfonylurea herbicides have been commercialized and others are being developed. All of these compounds apparently inhibit ALS by slow, tight-binding kinetics; that is, the initial K_i is significantly higher than the final, steady-state K_i. The herbicide molecule apparently binds reversibly to the ALS-FAD-thiamine pyrophosphate-Mg^{2+}-decarboxylated pyruvate complex and also competes for the second pyruvate binding site.

Sulfonylureas consist of an aryl group, a sulfonylurea bridge, and a nitrogen-containing heterocycle (Fig. 2). The aryl portion is generally phenyl substituted at the *ortho* position with either a halogen, a carboxy methyl, or a carboxy ethyl. Activity can be obtained with thiophene, furan, pyridine, or naphthalene groups as the aryl group. These groups must also be substituted at the *ortho* position for maximal activity. For highest activity, the heterocyclic group must be a symmetrical pyrimidine or triazine substituted in the 4 and 6 positions. Modification of the sulfonylurea bridge reduces activity.

The imidazolinones are noncompetitive with respect to pyruvate. Pyruvate must bind ALS before the herbicide can bind, and the binding is slow. The binding site may not overlap the second pyruvate binding site as with the sulfonylureas. Plants resistant to sulfonylureas may or may not be resistant to imidazolinones

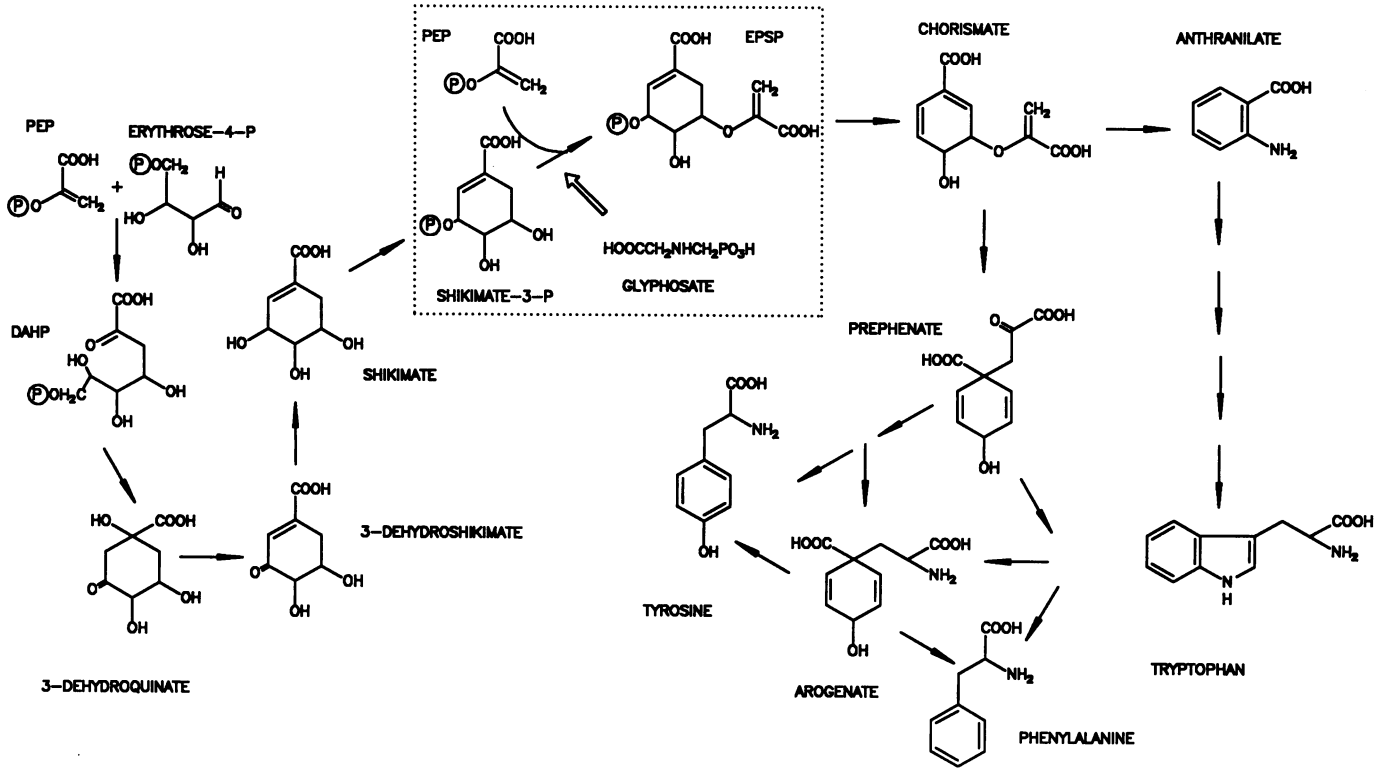


FIGURE 1. The shikimate pathway and the site of its inhibition by glyphosate. PEP = phosphocuolpyruvate, DAHP = 3-deoxy-D-*arabino*—heptulosonate-7-phosphate.

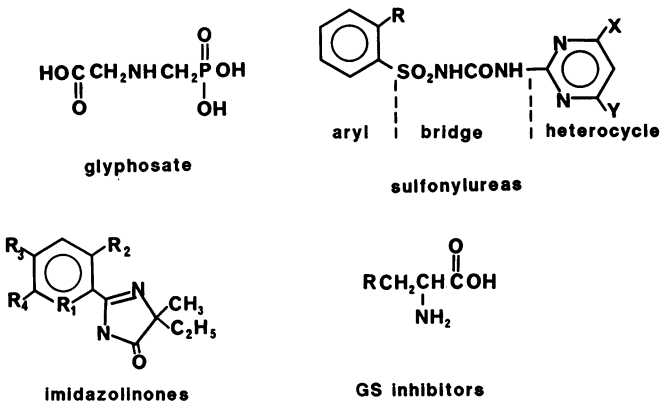


FIGURE 2. Chemical structures of herbicides and herbicide classes that inhibit amino acid biosynthesis. Sulfonylureas: R = carboxymethyl, carboxyethyl, chloro, or chloroethoxy; X and Y = methoxy, chloro, methyl, or ethoxy. Imidizolinones: R_1 = C or N; R_2 = carboxy, or carboxymethyl; R_3 = H or a phenyl carbon; R_4 = H, ethyl, methyl, or phenyl carbon. GS inhibitors: R = $CH_3POOHCH_2$- (glufosinate), $CH_3SONHCH_2$- (methionine sulfoximine), cyclic $CH_2NHCOCOH$- (tabtoxinine-β-lactam).

and vice versa. For maximal activity, imidazolinones should have the carbon adjacent to the carbonyl carbon of the imidazolinone ring substituted with both a methyl and isopropyl group (*8*). An aryl group with a carboxyl group in the *ortho* position should be attached to the carbon between the two nitrogens of the imidazolinone ring. The aryl group is generally a phenyl or pyridine group.

Although little is known of the pyrimidine inhibitors of ALS, they are analogues of hydroxyethyl-thiamine-pyrophosphate (HETPP), an ALS intermediate. Other analogues of HETPP are irreversible ALS inhibitors. To date, nothing has been published on the structural prerequisites for activity of this herbicide group.

Pyruvate oxidase and ALS have a remarkable level of amino acid sequence homology, and it has been suggested that the quinone-binding site of pyruvate oxidase and the herbicide binding site of ALS may have a common evolutionary origin (*9*). Ubiquinone homologues of the native pyruvate oxidase ubiquinone effectively inhibited ALS and will release radiolabeled sulphometuron methyl (a sulfonylurea herbicide) of the ALS molecule. The growing and diverse array of herbicidal ALS inhibitors and the apparently overlapping binding domains of these compounds at a quinone-binding site is similar to the situation with the plastoquinone binding site of the D-1 protein of photosystem II (PS II). This indicates that quinone-binding sites can be effectively bound by a relatively wide array of cyclic compounds.

GS is the first enzyme involved in assimilating inorganic nitrogen to produce an amino acid. It converts L-glutamic acid to L-glutamine in the presence of ammonia and ATP. Although numerous inhibitors of this enzyme are known, only two of these have been commercialized. Bialophos, a tripeptide fermentation product of *Streptomyces hygroscopicus*, is apparently metabolized to glufosinate in plants (*10*). Although bialophos is in commercial use in Japan, glufosinate (also known as phosphinothricin) (Fig. 2), the actual GS inhibitor, is being synthesized and marketed worldwide. Glufosinate is an irreversible inhibitor that competitively inhibits binding of glutamate to GS. Several other naturally occurring and synthetic analogues of glufosinate are known (Fig. 2) that are also excellent GS inhibitors. The inhibition of GS in plants that are reducing nitrate to ammonia

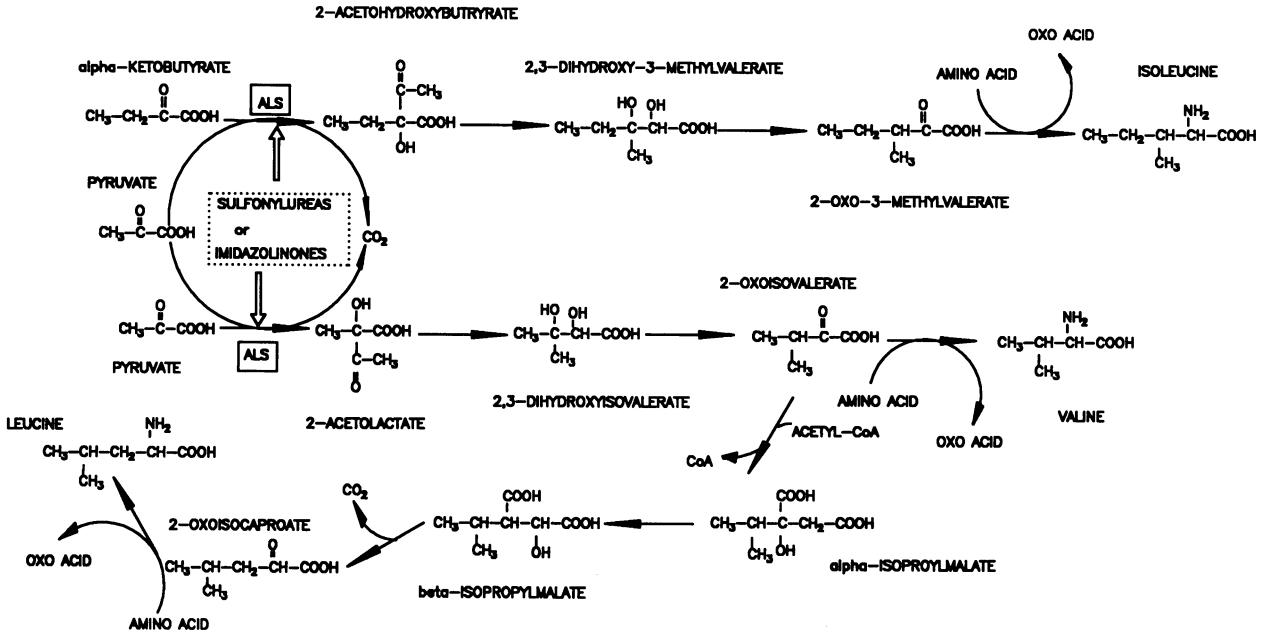


FIGURE 3. The branched chain amino acid pathway and the site (ALS) of its inhibition by sulfonylurea and imidazolinone herbicides.

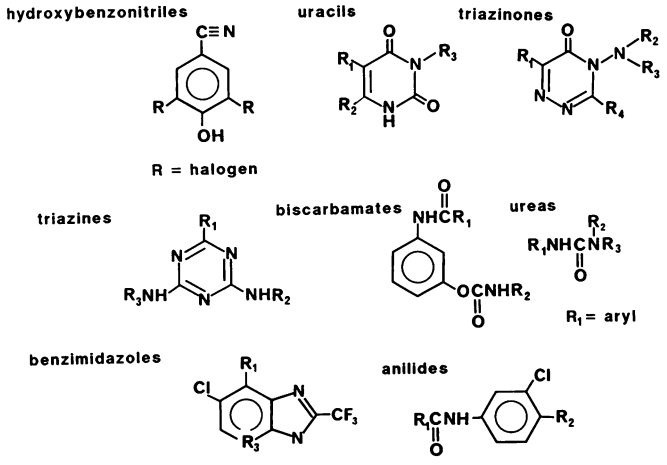
leads to accumulation of toxic levels of ammonia and rapid cellular collapse.

There are several common structural similarities shared by GS inhibitors related to glufosinate. These inhibitors are all substituted alanines with substitution at the terminal methyl group of alanine that can be quite varied in size and chemical properties (from a methyl sulfoximine to diazoacetyl).

Histidine biosynthesis in microbes is inhibited by the herbicide aminotriazole by inhibiting imidazole glycerol phosphate dehydratese (*7*). However, it is not established whether this is the herbicidal site of action in higher plants. In higher plants, amitrole is known to also inhibit carotenoid synthesis (*11*).

Photosynthesis

More herbicide classes act as photosynthetic inhibitors than by any other physiological process. The commercially available photosynthetic inhibitors all bind to the D-1, quinone-binding protein (formerly known as the Q_B protein) of photosynthetic electron transport and thereby block photosynthetic electron transport (*12*). Herbicidal chemical classes that bind this protein include anilides, benzimidazoles, biscarbamates, pyridazinones, triazinediones, triazines, triazinones, uracils, substituted ureas, quinones, hydroxybenzonitriles, and several unclassified heterocycles (*2*). A sampling of some of these chemical classes is given in Figure 4.



FIGURES 4. Chemical structures of some herbicide classes that inhibit photosystem II of photosynthesis by binding to the D-1, quinone-binding protein. Hydroxybenzonitriles: R = Cl, Br, or I; Uracils: R_1 = Br, Cl, or a cyclopenta carbon; R_2 = CH_3 or a cyclopenta carbon; R_3 = $CH(CH_3)$ (C_2H_5), $CH(CH_3)_2$, $C(CH_3)_2$, $C(CH_3)_3$, or cyclohexyl. Triazinones: R_1 = CH_3 or phenyl; R_2 = H_2 or $CHCH(CH_3)_2$; R_3 = SCH_3 or CH_3. Triazines: R_1 = SCH_3, OCH_3, or Cl; R_2 = C_2H_5 or $CH(CH_3)_2O_2$; R_3 = C_2H_5, $CH(CH_3)_2$ or $C(CH_3)_2CN$. Biscarbamates: R_1 = OC_2H_5, OCH_3, or $N(CH_3)_2$; R_2 = phenyl, $C(CH_3)_3$, or 2-methylphenyl. Ureas: R_1 = phenyls halogenated in the 3 and 4 positions, 3-chlorophenoxyphenyl, cyclooctyl, phenyl, and hexahydro-4,7-methanoindan-5-yl; R_2 = CH_3, thiadiazol, or 2-methylcyclohexyl; R_3 = OCH_3, CH_3, H, or C_4H_9. Benzimidazoles: R_1 = H or Cl, R_2 = N or C. Anilides: R_1 = $C(CH_2)CH_3$, cyclopropyl, $CH(C_3H_7)CH_3$, C_2H_5, or CH_3; R_2 = Cl or CH_3.

The native plastoquinone that normally binds the D-1 protein is thought to interact with histidine-215 and serine-264 of the protein. Herbicides that act at this site can roughly be classified into those that bind closer to the serine site or to the histidine site on the D-1 molecule. However, the differences are not that clear, with each herbicide group having slightly different binding characteristics. In higher plants, triazine-resistant biotypes of several weed species, in which serine-264 has mutated to glycine, are cross-resistant to those herbicides that also bind the serine binding site, but are not cross-resistant or are only weakly cross-resistant to those that primarily bind the histidine site (e.g., nitrophenols, pyridones) (*13*).

More has been published on the structure-activity relationships (SARs) of PS II inhibitors than any other group of herbicides. Quantitative structure-activity relationship (QSAR) analysis has provided detailed information on the size and position of substituents, electronic and steric parameters, and of the charge distribution of portions of the molecule directly involved in binding of each chemical class of PS II inhibitors (*14,15*). Generally PS II-inhibiting herbicides can be grouped by QSAR into diuron-types (serine-264) and phenol (histidine-215) types (Fig. 5).

Lipid Biosynthesis

Lipid synthesis has been strongly implicated in the mechanism of action of several herbicide classes (*16,17*); however, direct evidence of this site of action for a herbicide has only recently been obtained. Several laboratories have found that the aryloxyphenoxy alkanoic acids (*18–20*) and the cyclohexanediones (*18–22*) inhibit acetyl-CoA carboxylase. The enzyme present in most grasses is sensitive to these herbicides, while that in dicots is not. Generalized structures of these herbicide classes are shown in Figure 6. The actual biochemical mechanism of inhibition of this enzyme by these two herbicide classes is unknown.

The structural prerequisites for activity of the aryloxyphenoxy alkanoic acids appear to be an alkanoic (usually propionic) acid with a group containing more than one ring attached to an asymmetric, noncarbonyl carbon of the alkanoic acid. The ring structure attached to the acid must be attached to the other ring struc-

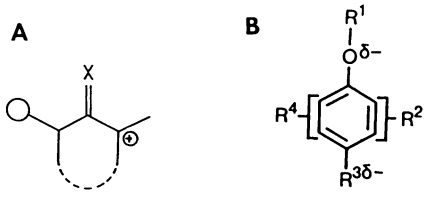


FIGURE 5. Generalized structural characteristics of diuron-type (*A*) and phenol-type (*B*) PS II inhibitors. *A*) O– = lipophilic group without strict steric requirements, X = O, S, or N. *B*) R^1 = H or electronegatively substituted or heterocycle, R^2 = slightly electron withdrawing group with strict steric requriements, R^3 = strongly electron withdrawing substituent, R^4 = lipophilic group without steric requirements.

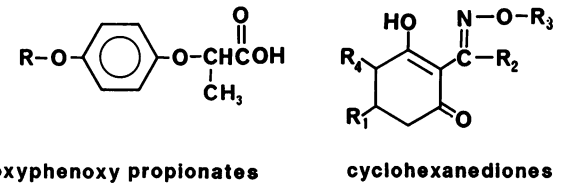


FIGURE 6. Chemical structures of herbicide classes that inhibit lipid synthesis by inhibiting acetyl Co-A carboxylase. Aryloxyphenoxypropionates: R = phenyl, pyridinyl, quinoxalinyl, or benzoxazoyl. Cyclohexandiones: $R_1 = (CH_3)_2$ or $CH_2CH(CH_3)SC_2H_5$; $R_2 = C_2H_5$, CH_2CHCH_2, or C_3H_7; $R_3 = C_3H_7$, C_2H_5, or $CH_2CHCHCL$; $R_4 = COOH_3$ or H.

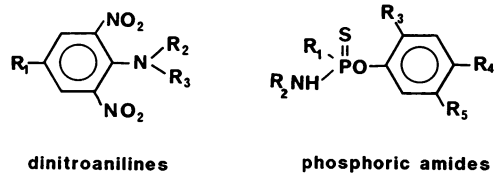


FIGURE 7. Chemical structures of herbical classes that inhibit cell division by interference with β-tubulin. Dinitroanilines: $R_1 = CF_3$, CH_3SO_2, or NH_2SO_2; $R_2 = C_3H_7$, C_2H_5, or H; $R_3 = C_3H_7$, C_2H_5, C_4H_9, CH_2CH_2Cl, or $CH(C_2H_5)C_2H_5$. Phosphoric amides: $R_1 = OCH_3$, CH_2Cl, or OC_2H_5; $R_2 = CH(CH_3)_2$ or $CH(CH_3)C_2H_5$; $R_3 = NO_2$ or Cl; $R_4 =$; Cl, CH_3, or H; R_5 = H or CH_3

ture(s) by a minimum distance of an ester linkage. The *R* enantiomer is much more active than the *S* enantiomer. Less is known of the structural requirements for herbicidal activity of the cyclohexanediones.

Cell Division

Many herbicides severely inhibit or disrupt cell division; however, the biochemical mechanism for the effect is not well understood for most of these compounds. The phosphoric amide and dinitroaniline herbicides (Fig. 7) are the only herbicide classes known to directly disrupt cell division by attacking a molecular site that is specific for cell division (*23*). These herbicides bind tubulin, the protein from which microtubules are composed. Microtubules are required for cell division and cell wall formation. Both of these herbicide classes inhibit polymerization of tubulin *in vitro*. Mutant biotypes of goosegrass are completely resistant to dinitroaniline and phosphoric amide herbicides (*24*). Tubulin is composed of an α and a β subunit. The β subunit is different in the herbicide-resistant goosegrass biotype (*25*). Nothing is known of the nature of the binding site of these herbicides on tubulin, and little has been published on the structure-activity relationships of herbicides that act at this site. Other potential molecular sites of herbicides for directly preventing mitosis are the microtubule-associated proteins and proteins of the microtubule-organizing centers.

Carotenoid Biosynthesis

Blockage of the terpenoid synthesis pathway at any point will result in inhibition of carotenoid synthesis, since carotenoids are formed quite late in the pathway. Carotenoids are more important for plant survival than any other products of this pathway because of the protection from photooxidation that they provide. Several important herbicides and herbicide classes (Fig. 8) inhibit this pathway. The substituted pyridazinones, *m*-phenoxybenzamides, fluridone, difunone, and 4-hydroxypyridines inhibit phytoene and phytofluene desaturase, a relatively uncharacterized enzyme or enzyme group (*11,26*). A separate structure-activity relationship is needed to characterize inhibition of phytoene desaturase for each of these herbicide families (*26*). For instance, a good correlation exists between lipophilicity and activity of substituted pyridazinones. Also, increasing *r* (Hammett electronic parameter) values in the carbon 4 position of the heterocycle of pyridazinones correlates positively with activity. In position 5, low *r* values enhance activity.

Aminotriazole amitrole inhibits cyclization of lycopene to form carotenes (*11*). The enzymology of this process is not well characterized. The 6-methyl pyrimidines such as dichlormate inhibit ζ-carotene desaturation (*11*). The isoxazolidinones are the only commercialized herbicides to inhibit carotenoid synthesis at a point earlier in the terpenoid pathway than phytoene desaturase (*27*). Clomazone, a representative of this class, inhibits either isopentenyl pyrophosphate isomerase or prenyl transferase (*28*). Little is known of the enzymes of the terpenoid pathway in plants.

For best activity, the isoxazolidinones must have the carbonyls in the 3 and 5 positions or in the 3 position with an aryl group attached at the 2 position (*29*). A (chlorophenyl)methyl aryl group provides optimal activity. A dialkyl substitution in the 4 position of the isoxazolidinone ring is a requirement for activity and a halogen in the 2 position of the phenyl ring further enhances activity. The most active isoxazolidinones are not good herbicides because of rapid degradation in the

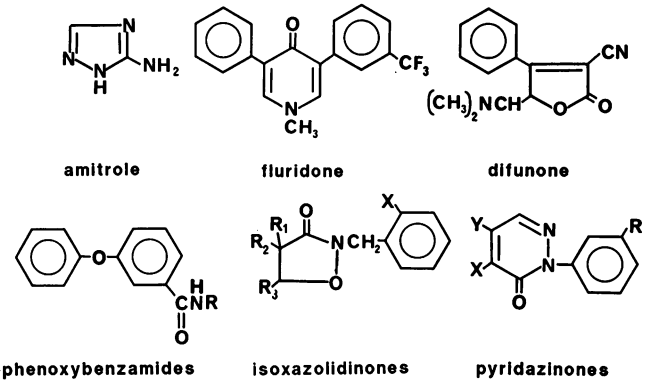


FIGURE 8. Chemical structures of herbicides and herbicide classes that inhibit carotenoid synthesis. *m*-Phenoxybenzamides: the R group can be a branched or linear alkyl group; Isoxazolidinones: R_1-R_2 pairs = H and C_3H_5, H and H, CH_3 and CH_2Cl, or H and CH_3; $R_3 = OCH_3 > Cl > OH > SCH_3$; X = Cl > Br > F > CH_3; Pyridaziones: X = Br or Cl; Y = NH_2, $NHCH_3$, or $N(CH_3)_2$, R = CF_3 or OCF_3.

field. For this reason, only the 3-isoxazolidinones have been commercialized.

Photobleachers

Several herbicide classes cause rapid photobleaching of green tissue. One group of these compounds is reduced by photosystem I (PS I) to a radical that, in turn, reduces molecular oxygen to superoxide radical. Thus, the herbicide acts in concert with PS I to catalytically generate large amounts of superoxide radical. When the plant tissue cannot defend itself from excess superoxide radical, rapid lipid peroxidation and photobleaching occur. Only one group of herbicides with this mode of action has been commercialized—the bipyridyliums, including paraquat and diquat (*30*). Other chemical classes that act by this mechanism, such as the heteropentalenes (*31*), have been patented. Fedtke (*2*) summarized the chemical requirements for a molecule to generate superoxide radical via PS I: the redox potential should be between −300 and −714 mV; a stable, water-soluble radical should be formed upon one electron transfer; and the radical must be able to transfer its electron to molecular oxygen (i.e., very stable radicals are not herbicidally active).

Several other herbicide groups cause photobleaching by mechanisms that are not dependent on photosynthesis (*32–34*). Figure 9 provides a sampling of photobleachers that apparently act by photosynthesis-independent mechanisms. The most important of these herbicide groups are the *p*-nitrodiphenylethers (DPE), the oxadiazoles, and the *N*-phenyl imides. None of these compounds act directly as photosensitizing pigments. Although their mechanism of action has remained a mystery for two decades, recent findings from several laboratories indicate that the DPEs as well as other herbicide classes with similar activity cause massive accumulation of protoporphyrin IX, a potent photosensitizer (*33–36*). They cause this accumulation by inhibition of protoporphyrinogen oxidase, resulting in the uncontrolled autoxidation of protoporphyrinogen to protoporphyrin IX (*37,38*).

Although structure-activity information is available on DPEs (*39*) and *N*-phenyl imides (*40,41*), there are no obvious chemical properties common to these two chemical classes that cause the accumulation of protoporphyrin IX in plants.

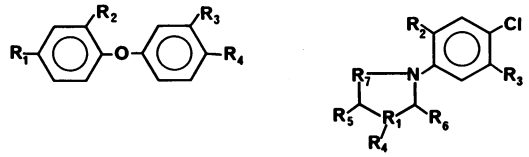


FIGURE 9. Chemical structures of three photobleaching herbicide classes. Diphenyl ethers: R_1 = Cl or CF_3; R_2 = Cl or NO_2; R_3 = $COOCH_3$ or $COOCH_2CH_3$; R_4 = Cl or NO_2. N-phenyl imides: R_1 = C; R_2 = H or F; R_3 = H or an alkoxy group; R_4 and R_5 = phenyl carbon, ethyl, or methyl; R_6 = O or S; R_7 = CO, CS, or CNCOOEt. Oxadiazoles: R_1 = O; R_2 = Cl or NO_2; R_3 = $COOCH_3$; R_4 = H; R_5 = $C(CH_3)_2$; R_6 = O; R_7 = NH.

Other Sites of Action

Only two other molecular sites of action of herbicides are known. The herbicide asulam inhibits folate synthesis by inhibiting dihydropteroate synthase (*42*). While this may be the primary site of herbicidal action, there may also be a second site associated with cell division. Cellulose synthesis is specifically inhibited by the herbicide dichlobenil; however, its molecular site of action is still unknown (*43*). Photoaffinity labeling of cotton fiber proteins with a photoaffinity dichlobenil analogue resulted in specific labeling of an uncharacterized 18 kD protein.

Other Factors That Affect Activity

For the success of a herbicide, many chemical factors other than efficacy at the molecular site of action must be considered. Chemical structures are sought whose properties lead to optimization of absorption, translocation, selectivity, environmental stability, and efficacy. Sometimes these requirements can be obtained within one molecular configuration, and in other cases the molecular constituents that optimize desirable traits other than activity at the site of action can be designed to be enzymatically cleaved by the target plant.

Absorption and Translocation

It is well known to those who screen potential herbicides that the relative efficacies of herbicides can change considerably when absorption and translocation barriers are removed. For instance, an anilide herbicide, MT-5950, was much less effective than atrazine in inhibiting PS II when applied to intact plants, whereas it was much more effective than atrazine when leaf discs were floated on the herbicide, thus eliminating cuticular absorption barriers (*44*).

It is highly desirable that herbicides be translocated to all parts of a plant. Herbicides that are not translocated will kill only those portions of the plant to which they are applied, leaving the other parts of the plant to regenerate a competitive weed. Thus, a phloem-mobile analogue (generally weakly acidic at physiological pH) of a herbicide with less activity at the site of action may be more desirable than a more highly active form that is poorly translocated (often a nonionic and highly lipophilic form).

Bioactivation

Several herbicides are applied to plants in a chemical form that is inactive at the molecular site of action. This applied proherbicide form may be more stable, more readily absorbed and/or translocated, more selective, or more easily synthesized than the active form. Once inside the plant cell, the proherbicide must be metabolized

Table 1. Summary of the known molecular modes of action of herbicides.

Physiological site	Molecular site	Herbicide class
Amino acid synthesis	EPSP synthase	Glyphosate
	Acetolactate synthase	Sulfonylureas
		Imidiazolinones
	Glutamine synthetase	Glufosinate
Photosynthesis	D-1 quinone-binding protein	Triazines
		Anilides
		Substituted ureas
		Biscarbamates
		Benzimidazoles
		Uracils
		Quinones
		Hydroxynitriles
Bleaching	Photosystem I	Bipyridyliums
		Heteropentalenes
	Protoporphyrinogen oxidase	Diphenyl ethers
		Oxadiazoles
		N-phenyl imides
Lipid synthesis	Acetyl-CoA carboxylase	Aryloxyphenoxy propionates
		Cyclohexanediones
Carotenoid synthesis	Phytoene desaturase	Substituted pyridazinones
		Fluridone
		m-Phenoxybenzamides
		4-Hydroxypyridines
	Lycopene cyclase	Aminotriazole
	ζ-Carotene desaturase	Dichlormate
	IPP isomerase and/or prenyl transferase	Isoxazolidinones
Cell division	β-Tubulin	Dinitroanilines
		Phosphoric amides
Cellulose synthesis	Cellulose synthase?	Dichlobenil
Folate synthesis	Dihydropteroate synthase	Asulam

to the active form of the herbicide (*45*). For instance, most of the aryloxyphenoxy alkanoic acids are applied as methylesters that have no effect on acetyl-CoA carboxylase. Once inside the plant, the ester is hydrolyzed to the active acid form of the herbicide. The phenoxy alkanoic acids such as 2,4-D are also generally applied as esters. In both of these cases, the ester form is much more readily absorbed through plant cuticles than the acid form. Other examples of herbicides that are bioactivated are benzadox, which is converted to the potent aminotransferase inhibitor, aminooxyacetic acid (*46*); bialophos which is metabolized to glufosinate; and methazole, which is converted to a substituted urea herbicide. In the past, herbicides have been discovered and marketed with no knowledge of whether the compound is a proherbicide or is active in the unmetabolized state. Differential bioactivation can be the basis for target species selectivity.

Environmental and Metabolic Stability

A herbicide that is highly active and has good properties in the laboratory or greenhouse may be unsuitable for commercial use because of an undesirable environmental half-life. For instance, the most active isoxazolidinones do not persist in the field sufficiently long to be effective herbicides (*29*). Conversely, compounds with an extremely long half-life may not be useful because of movement to groundwater and incompatibility with subsequent rotated crops. Similarly, compounds that are quite active at the site of action may be poor herbicides because they are readily metabolized to inactive derivatives by target organisms. These undesirable properties can be altered by molecular changes that have nothing to do with the mechanism of action of the molecule and usually result in lower herbicidal activity.

SAR and Mode of Action of Herbicides

Virtually all SAR information that is available for herbicides is semiempirical. That is, it is largely unrelated to the molecular site of action, often being developed with no knowledge of the site of action. In fact, many SAR and QSAR studies have been published with no indication of the type of herbicidal damage caused or how it was determined. In several cases, herbicide classes can affect more than one site of action and separate SAR and QSAR studies have been conducted for each site of action. For instance, substituted pyridazinones can inhibit phytoene desaturase and PS II. They can also alter lipid synthesis. The SARs for each of these sites are different (*26,47*). Similarly, diphenyl ether compounds can inhibit carotenoid synthesis, ATP formation, and photosynthetic electron transport, or induce membrane peroxidation by causing massive accumulation of protoporphyrin IX (*35,39*). The SARs for each of these sites of action are different. Thus, for many compounds that belong to known chemical classes of herbicides, predicting herbicidal activity and type of herbicidal activity from our SAR data base can be quite

complex. To predict herbicidal activity and/or mechanism of action of a compound that does not belong to a known herbicide class is generally a wild guess. The problem is compounded by extra molecular baggage that may be involved in altering absorption, translocation, environmental stability, or bioactivation. Additional SARs using each of these parameters as an end point can be developed.

Summary

The exact molecular site of action for many herbicides remains unknown. Still, the known molecular sites represent a large array of biochemical processes (Table 1). Nevertheless, for many of these known molecular sites, chemical families representing a diversity of structures are effective inhibitors. Although many additional potential sites of action have yet to be exploited, knowledge of both synthetic inhibitors and phytotoxins of microbial origin (*10*) indicate that these sites have great future potential. Numerous compounds are very active in inhibiting a molecular process *in vitro*, but they are not active herbicides because of absorption, translocation, or metabolism properties. Furthermore, some herbicides and herbicidal chemical classes act at more than one molecular site of action. Thus, the potential number of sites of action for herbicides is quite large. In the open literature, the number of structure-activity relationships of herbicides at the molecular level is quite limited. The picture is further clouded by the fact that the structural requirements for optimizing absorption, translocation, stability, and other desirable properties can complicate the relationship between a usable herbicide and its molecular site of action.

To date, there is no convincing evidence that any herbicidal class has been targeted to attack a new site of action. In fact, the exact molecular target site of all major groups of herbicides that have been commercialized have been discovered after patenting, if at all. Thus, it is clear, that our ability to predict the site of action of a herbicide class, based on structure of the herbicide as applied in the field, is quite limited. Our present capabilities are based on semiempirical knowledge of structure-activity of known compounds and of structures of metabolic intermediates of plant biochemical pathways.

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